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Capsaicin-induced effects on *c-fos* expression and NADPH-diaphorase activity in the feline spinal cord

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Abstract

The distribution of *c-fos* expression and NADPH-diaphorase reactivity in the cervical and lumbar segments after stimulation of the vanilloid receptors in the dorsal neck muscles with capsaicin was studied in cats anaesthetized with α-chloralose. After the unilateral intramuscular injection of capsaicin, the mean number of Fos-immunoreactive neurons detected with an avidin–biotin–peroxidase technique was significantly increased in the superficial laminae (I), neck of the dorsal horn (V), and area around the central canal (VII) within both the cervical and lumbar spinal cord. Most Fos-immunoreactive neurons in the cervical spinal cord were giant and small cells. The widespread distribution of Fos-immunoreactive cells throughout the cervical cord within the intermediate zone (VII) coincided with the sites of localization of last-order premotor interneurons and cells of origin of inter-segmental crossed and uncrossed descending propriospinal pathways to the lumbar spinal cord. Fos-immunoreactive neurons were co-distributed with nitric oxide-generating cells at both levels of the spinal cord, although the double-labeled cells were not observed. In conclusion, the analysis of *c-fos* expression and NADPH-diaphorase reactivity shows that stimulation of vanilloid receptors in the neck muscles can initiate distinctive neuronal plasticity in the cervical (C1–C8) and lumbar (L1–L7) segments, and confirms the anatomical and functional coupling of both regions during processing of nociceptive signals from the dorsal neck muscles.

Keywords: c-Fos; Nitric oxide synthase; Pain; Plasticity; Spinal cord

1. Introduction

Capsaicin is prototypic vanilloid receptor agonist which is now widely used as pharmacological tool to induce experimental models of acute skeletal muscle pain in animal and human (Sluka and Willis, 1998; Herbert and Holzer, 2002), as well as the therapeutic agent in clinical trials (Watson et al., 1993). Although capsaicin initially stimulates glutamate and neuropeptide release, it also induces a sustained inhibitory effects that are manifested in its analgesia and anti-inflammatory actions (Winter et al., 1995). The vanilloid receptor (TRPV1) is molecular target responding to noxious heat and protons (Caterina, 2003), and expressed in predominant (85%) of substance P-containing primary afferent fibers. In skeletal muscles such type of receptors could be activated during myositis accompanied with increase of tissue temperature, sustained contraction, hypoxia or injury. Intramuscular injections of capsaicin in humans have been shown to induce a deep pain of typical intensity profile, and that is characterized by referred pain and hyperalgesia (Graven-Nielsen and Mense, 2001). It was shown that capsaicin could stimulate predominantly group IV muscle afferents (Kaufman et al., 1982), and capsaicin-sensitive fibers activate spinal inhibitory pathways which attenuate motoneuronal output during muscle fatigue (Pettorossi et al.,

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1999; Kalezic et al., 2004a; Kostyukov et al., 2005). However, the spinal circuits that involved into nociceptive processing followed by activation of vanilloid receptors in the neck muscles are not still detailed. Immunohistochemical method of *c-fos* expression as a marker of neuronal activation can be fruitful to study the intra- and inter-segmental pathways involved into nociception. It was shown that the chemical or mechanical activation of Aδ and C (group III and IV) primary muscle afferents induced *c-fos* expression in the spinal neurons (Harris, 1998; King and Apps, 2000; Pilyavskii et al., 2001; Buritova and Besson, 2002).

Capsaicin activates a number of biochemical systems and increases concentration of nitric oxide (NO) in tissue (Bauer et al., 1995). It is well documented that nitric oxide synthase (NOS) that is presented in some spinal neurons can be expressed in a much greater number of central neurons and, additionally, in glial cells during acute pain, hyperalgesia, inflammation or muscle fatigue development (Herdegen et al., 1994; Vizzard et al., 1995; Maisky et al., 2002). Recent findings demonstrate that endogenous NO may modulate the activity of group IV muscle afferents (Arbogast et al., 2001; Urch and Dickenson, 2003), and may be involved in the mechanisms of capsaicin-induced nociceptive response (Sakurada et al., 1996).

In an attempt to further elucidate the spinal circuits that convey the signals from muscle nociceptors, we set out to test the hypothesis that persistent stimulation of vanilloid receptors in the feline dorsal neck muscles by capsaicin can induce distinctive patterns of Fos-immunoreactivity and NADPH-diaphorase reactivity within both the cervical and lumbar spinal cord. Neuroanatomical data about pathways from capsaicin-sensitive neck muscle afferents to the cervical and lumbar spinal cord are presented in this study preliminary data were published elsewhere (Kalezic et al., 2004b).

2. Materials and methods

2.1. Experimental groups and pain stimulation protocol

Twelve cats of either sex (2.5–3.0 kg) were used in these experiments: (1) control group, without preceding injections ($n=3$); (2) vehicle-injected group ($n=3$); (3) capsaicin-treated group ($n=6$). The pain stimulation protocol and the handling of the animals were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Animals of all experimental groups were anaesthetized (α-chloralose 50 mg/kg i.p.). In animals of a treated group, capsaicin (Sigma) dissolved in Tween 80 (7%) and isotonic saline (93%) was injected in dose of 200 μg into each 10 sites along the rostro-caudal extent of the right rostral parts of *mm. trapezius* and *splenius;* and in animals of a vehicle injected group only vehicle (Tween 80 and saline) was used. After injection, the needle was kept in place for 1–2 min in order to prevent capsaicin from leaking into subcutaneous tissues.

2.2. Perfusion

The animals were perfused 2 h after the end of the capsaicin or vehicle injections. The control and experimental cats were deeply anaesthetized (sodium pentobarbital 75 mg/kg i.p.) and perfused through the ascending aorta with 0.9% physiological saline (500 ml) followed by fixative solution (1500 ml) containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Blocks of the cervical and lumbar spinal cord were quickly removed, postfixed in the same fixative overnight and cryoprotected in phosphate-buffered sucrose at 4 °C for 48 h. Frontal frozen sections of the spinal cord (30 μm thick) were cut. About 50–80 serial sections from each cervical (C1–C8) and lumbar (L1–L7) segment were collected in 15 wells with cold phosphate-buffered saline (PBS) (0.01 M phosphate buffer containing 0.9% NaCl), to be processed immunohistochemically and histochemically.

2.3. Fos immunohistochemistry and NADPH-diaphorase histochemistry

Fos immunoreactivity (i.e. labeled nuclei of Fos-immunoreactive spinal neurons) ipsi- and contralateral to the stimulated side was detected according to a standard avitin–biotin–peroxidase technique (Hsu et al., 1981), using a rabbit polyclonal antibody directed against c-Fos protein (Oncogene Research, Ab-5, USA) and a commercial kit (ABC Kit, Vector Laboratories, PK 4001, USA). Briefly, following several rinses in PBS, the sections were placed in PBS containing 3% normal goat serum (Sigma) with 0.3% Triton X-100 (Sigma) for 30 min at room temperature. Free-floating sections were then incubated for 48 h at 4 °C in primary antiserum against c-Fos protein diluted (1 : 4000) in PBS containing 3% normal goat serum and 0.4% Triton X-100. The sections were then incubated in biotinylated goat antirabbit immunoglobulin G (1 : 200) and avidin–biotin complex (ABC) using a standard protocol. Fos-immunoreactive nuclei were visualized with nickel-intensified 3,3′-diamino-benzidine tetrahydrochloride (DAB, Sigma). Sections were reacted in a 0.05 M Tris–buffer (pH 7.4) containing 0.035% DAB, 0.2% nickel ammonium sulphate and 0.005% hydrogen peroxide for 10 min at room temperature to produce a purple-black reaction product. The sections were washed in PBS and mounted on gelatine-subbed slides, air-dried, cleared in xylene and finally cover-slipped. Fos-immunoreactive neurons and glial positive cells were detected as black nuclei. To evaluate the possibility of double labeling of Fos immunoreactivity and NADPH-diaphorase reactivity that manifested NOS-containing spinal neurons in cats (Dun et al., 1993), half of the immunostained sections from cervical and lumbar segments were additionally incubated in 0.1 M phosphate buffer (pH 7.4) containing 0.3% Triton X-100, 0.2 mg/ml nitroblue tetrazolium (Sigma) and 0.5 mg/ml β-NADPH tetra-sodium salt (Sigma), at 37 °C for 30–60 min (Vincent and Kimura, 1992). NADPH-diaphorase-reactive neurons were easily detected as light-

blue cells with unstained nuclei, especially at high magnification (×400).

2.4. Data analysis

Fos-immunoreactive neurons and NADPH-diaphorase-reactive cells were counted in the cervical (C1–C8) and lumbar (L1–L7) spinal segments. Up to 12 stained sections (Fos immunoreactivity) and up to 15 stained sections (NADPH-diaphorase reactivity) or double-stained sections were taken from each segment per cat and analyzed. A mean number±S.E.M. of labeled cells per section (30 μm) were counted in laminae I–X of the gray matter on the ipsi- and contralateral side. The numbers of the stained neurons on both sides of the spinal segments, or within different segments, from the cats of the capsaicin-treated group were compared using two-way statistical analysis of variance (ANOVA). The factors of variation included two conditions (contra- and ipsilateral sides and seven levels C2–C8 and L1–L7). Newman–Keuls' post hoc analysis was used when a significant difference was found. Values of $P<0.05$ were considered statistically significant.

3. Results

In the intact cats, the basal level of *c-fos* expression on the both sides of the cervical and lumbar spinal cord was very low (one or two Fos-immunoreactive neurons per 30 μm thick section). Although, in the vehicle-injected cats the number of Fos-immunoreactive neurons was found to increase bilaterally in the rostral cervical and lumbar segments as response to injection-related pain. For example, in the C3/C4 and L4/L5 segments, the mean number of labeled neurons rose to 5.8±0.9 ($n=3$) and 3.5±0.4 ($n=3$) cells, respectively.

3.1. Capsaicin-induced c-fos expression in the cervical spinal cord

As compared to the vehicle-injected animals, the most distinctive increase in mean number of labeled neurons per section were found in the cervical C1–C7 segments in the capsaicin-injected side, with prevalence of cell accumulation (40.2±4.1, $P<0.01$) at the C3 segment. The transverse distribution of labeled cells was clearly nonuniform in the both dorso-ventral and medio-lateral direction in all studied segments (Fig. 1). First, the statistically significant number of labeled neurons occurred in laminae I, V and VII of the stimulated (right) side of the cervical cord. Second, the giant and small immunoreactive nuclei were comprised in the lateral half of the superficial dorsal horn, and revealed throughout the deeper part (neck) of the dorsal horn and intermediate gray matter (Figs. 2 and 3). In the C5–C7 segments Fos-immunoreactivity declined in all laminae. However, predominant number of labeled cells was in laminae I and VII. It is needed to note that the nucleus proprius of the dorsal horn (laminae III and IV), receiving projections from large-diameter primary afferents, contains a very few staining neurons on both sides. The difference in mean number of Fos-immunoreactive neurons on the contralateral side in animals of the capsaicin-treated group and on either side of the vehicle-injected group was not statistically significant ($P>0.05$). A few Fos-immunoreactive motoneurons were also found within motor nuclei of the ventral horn (Figs. 1F and 3).

Fos-immunoreactive nuclei of the labeled neurons appeared as round or elongated in shape were surrounded with intensely labeled elements (conceivably, the nuclei of glial-like cells) <10 μm in size of irregular (vermicular, spiroid or hooked) in form. More significant capsaicin-related glial immunoreactivity was seen predominantly in the superficial laminae (I, IIo) and neck of the dorsal horn (lamina V), i.e., in the zones of termination of high-threshold muscle afferents (Fig. 1B, C, E, H). Although the labeled glial cells were mainly located on the ipsilateral side, clear glial activation also occurred within the superficial dorsal horn on the contralateral side of the C1–C8 segments.

3.2. NADPH-diaphorase reactivity in cervical cord

Histochemical staining revealed that NADPH-diaphorase-reactive cells within the cervical spinal cord were typically designated by a specific blue-staining of the neuronal soma and processes, but the nuclei were free of staining (Fig. 2). In the both vehicle-injected and capsaicin-treated groups of cats, the reactive neurons were displayed bilaterally mainly within superficial laminae (I, IIo), neck of the dorsal horn (lamina V) and medial part of lamina VII (Figs. 2 and 3). Following capsaicin injection, most of stained neurons were found in the C5–C8 segments with predominance in the ipsilateral lamina I ($P<0.01$). No significant changes in the number of positive neurons were detected in laminae II in the capsaicin-treated cats (Table 1). Fig. 2 shows that NADPH-diaphorase-reactive cells were often intermixed with Fos-immunoreactive neurons. Many Fos-immunoreactive neurons were located in close proximity or in opposition to NADPH-diaphorase-reactive elements within superficial laminae (I, IIo) and the neck of the dorsal horn (V, VI). The *c-fos* expression in the NADPH-diaphorase-reactive neurons at the cervical level in the present study was not found (Fig. 2A, B).

3.3. Capsaicin-induced c-fos expression and NADPH-diaphorase reactivity in lumbar spinal cord

In the lumbar spinal cord, *c-fos* expression was detected in all segments studied. The mean number of Fos-immunoreactive neurons rose progressively from L1 to L6. However, in the lumbar spinal cord, the total number of labeled neurons was just about a third of the number in the cervical cord. For example, the mean number of Fos-

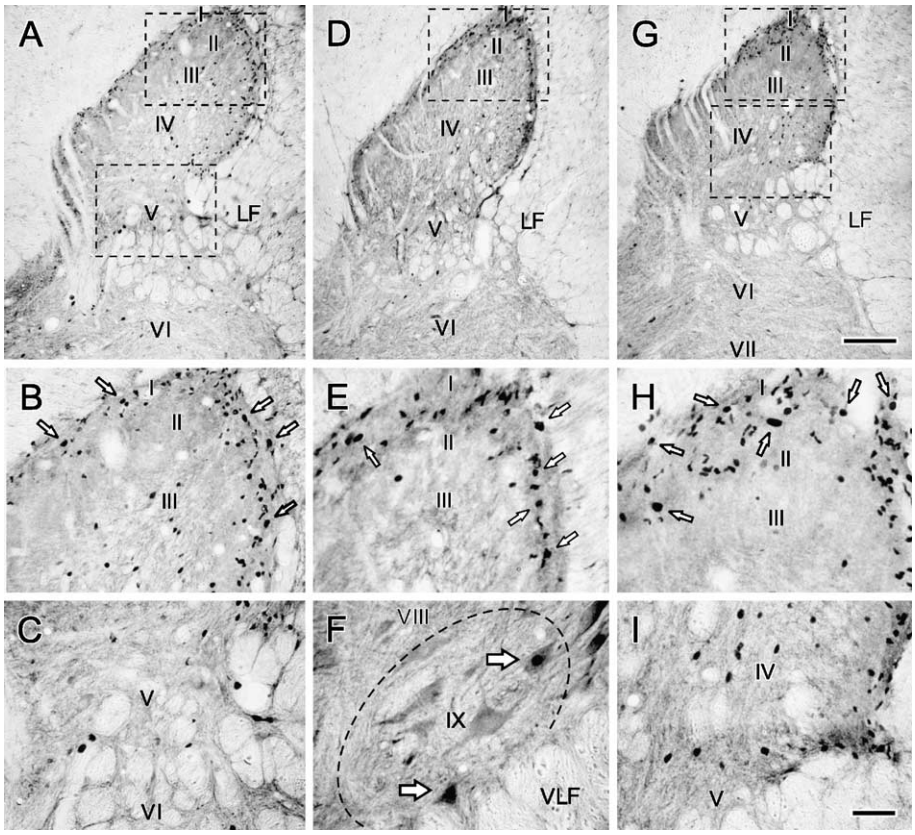

Fig. 1. Photomicrographs of immunohistochemically stained sections of the cervical spinal cord demonstrating *c-fos* expression following capsaicin injection into neck muscles in cat. Fos-immunoreactive neurons (white arrows) and staining of glial-like cells (irregular in form) in the dorsal and ventral horn in the C3 (A–C), C4 (D–F) and C5 (G–I) segments, in the ipsilateral (right) side to capsaicin injection. The dashed boxes in A, D and G delimitate the areas presented at higher resolution in (B, C), (E) and (H, I), respectively, showing the large labeled nuclei in lamina I (white arrows) in the dorsolateral part and the neck of the dorsal horn. Labeled motoneurons in lamina IX (large white arrows in F). LF and VLF, lateral and ventrolateral funiculi, respectively. Scale bars: 200 and 50 μm for (A, D, G) and (B, C, E, F, H, I), respectively.

immunoreactive neurons at the main focus of their localization (L4–L6 segments) was 9.8±0.9 positive cells vs. 40.2±4.1 in the C3–C5 segments. The difference between the relative proportions of Fos-immunoreactive neurons on the ipsilateral and the contralateral sides in the L1–L6 segments was not significant (Figs. 4 and 5). Only at the L7 level, Fos-immunoreactive cells prevailed at the contralateral side, and this difference was statistically significant ($P<0.05$).

In both experimental groups of animals (vehicle- and capsaicin-treated), the maximal density of NADPH-diaphorase-reactive neurons in the lumbar spinal cord was detected in the L5–L7 segments (Fig. 5). The positive cells were predominantly localized in the superficial laminae, neck of the dorsal horn and lamina VII at both ipsi- and contralateral sides. The mean numbers of positive neurons in capsaicin-treated and vehicle-injected animals were different. Table 1 demonstrates the significant exceeding of stained cells detected in the laminae I and II of the L6 and L7 segments in the treated cats ($P<0.05$). In contrast to the cervical spinal cord, a few NADPH-diaphorase-reactive motoneurons were found within the ventral and lateral motor nuclei in the ventral horn (Fig. 5). Although

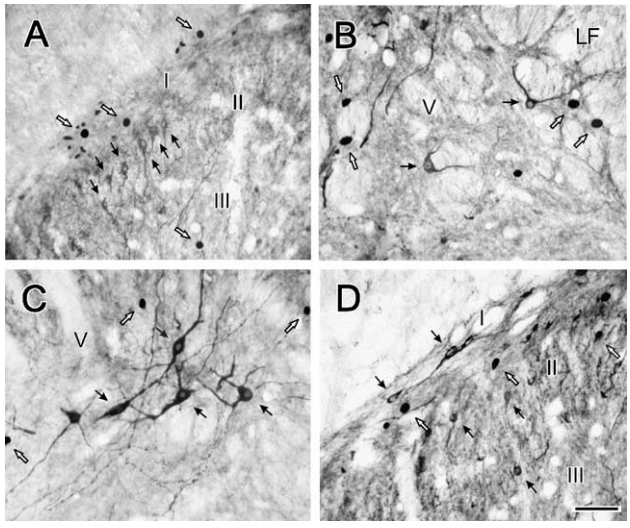

Fig. 2. Co-distribution of Fos-immunoreactive and NADPH-diaphorase-reactive neurons within medial part of superficial laminae (A, D) and lateral part of neck of the dorsal horn (B, C) in the stimulated side. Elongated and round labeled nuclei of spinal neurons (white arrows) are intermixed with fusiform and multipolar NADPH-diaphorase-reactive cells with blue-staining somata and dendrites (black arrows) within laminae I–V of the dorsal horn in segments C4 (A, B), C5 (C), and C6 (D). Scale bar: 50 μm.

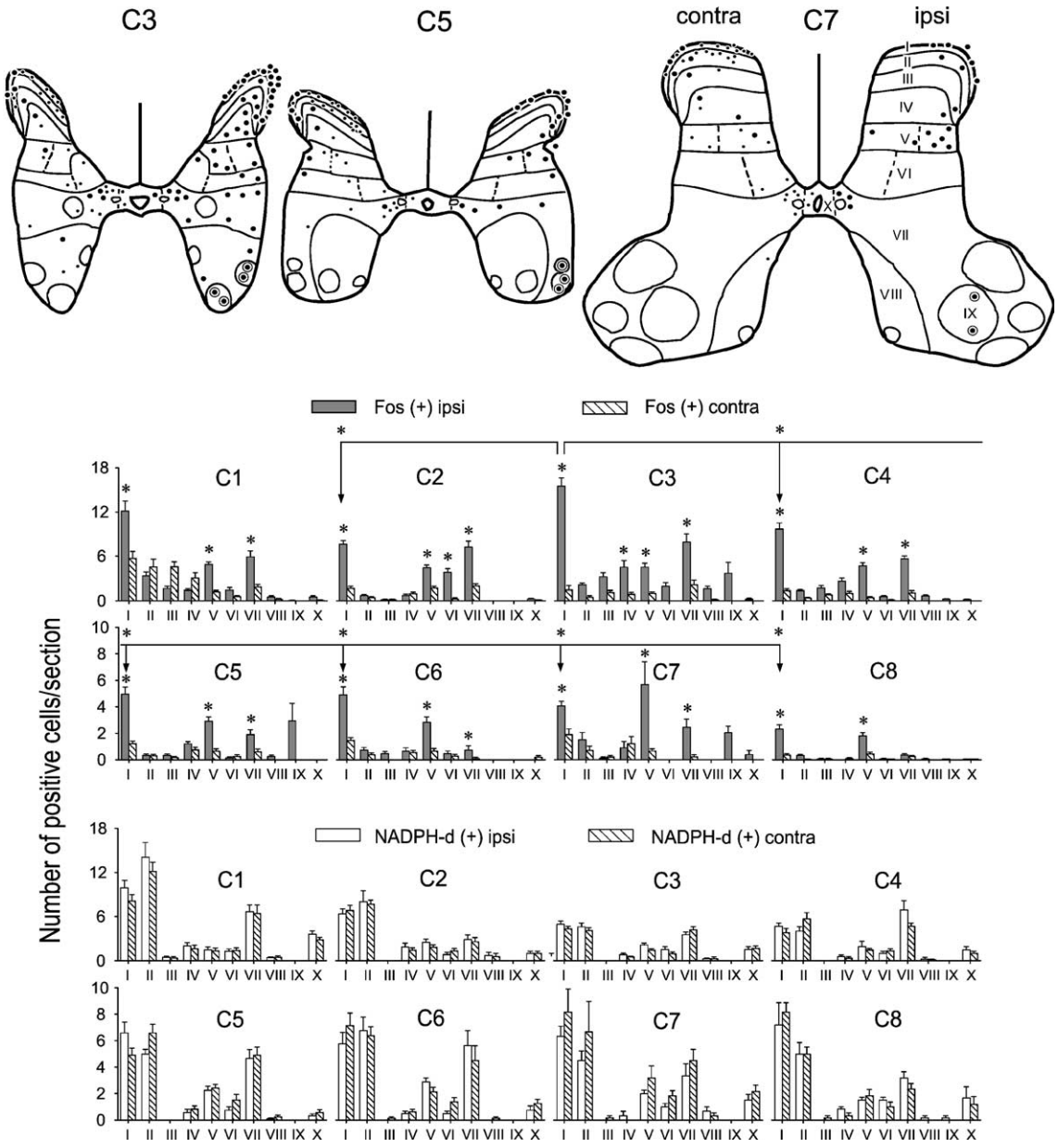

Fig. 3. Drawings (upper row) illustrating co-distribution of Fos-immunoreactive neurons and NADPH-diaphorase-reactive cells, and bar graphs (lower panel) of their mean numbers in ipsi- and contralateral sides in cervical C1–C8 segments after capsaicin injection into neck muscles. Mean numbers (±S.E.M.) of stained cells per section were defined in six treated cats. Note the ipsilateral prevalence of Fos-immunoreactive neurons (large dots in upper drawings) in the dorsal horn (laminae I–VI), area around the central canal (laminae VII), motor nuclei (circles in lamina IX) of the C3, C5 and C7 segments, and bilateral distribution of NADPH-diaphorase-reactive cells in the same segments; NADPH-diaphorase-reactive cells (small dots) are shown only in the contralateral side of the drawings. Asterisks in the lower panel indicate significant differences in the numbers of labeled cells in the ipsilateral vs. contralateral side for a given layer, when asterisks are placed on the columns; in the ipsilateral side for a given layer and a given segment vs. another segments, when they are placed on the lines (*$P<0.05$).

Fos-immunoreactive and NO-generating neurons intermingled within the superficial laminae I and IIo, the lateral part of lamina V, and the area around the central canal, the double-labeled cells were never seen.

4. Discussion

The findings presented here demonstrate that the selective stimulation of vanilloid receptors in the dorsal neck muscles initiates the neuronal plasticity in both cervical and lumbar spinal segments. The plastic changes are characterized by a significant increase in the number of Fos-immunoreactive neurons in the C1–C8 segments, predominantly on the ipsilateral (injected) side. However, in the lumbar segments, there is a marked bilateral rise in the number of labeled cells. The defined labeling of the interneurons in the lumbar segments is in line with data suggesting that cervical neurons may contribute to the polysynaptic activation of other neurons along the spinal

Table 1
Capsaicin- or vehicle-induced effects on the spinal NADPH-diaphorase activity

		Capsaicin		Vehicle	
		Ipsi	Contra	Ipsi	Contra
C3	I	4.93±0.44*	4.33±0.34	2.33±0.49	1.58±0.31
	II	4.6±0.52	4.06±0.38	3.91±0.39	3.58±0.49
C4	I	4.66±0.44*	3.77±0.61	1.5±0.31	2.4±0.58
	II	4.0±0.64	5.66±0.86	2.66±0.41	3.4±0.61
C5	I	6.58±0.81	4.92±0.53	3.69±0.75	2.36±0.47
	II	5.0±0.34*	6.58±0.66	2.83±0.46	2.36±0.66
L5	I	6.3±0.83*	6.5±0.68	1.12±0.29	0.62±0.26
	II	6.2±0.79*	5.8±0.55	1.87±0.39	1.0±0.27
L6	I	17.37±0.94*	14.71±1.56	3.66±0.66	3.87±0.74
	II	6.0±0.42*	6.37±0.53	2.55±0.41	3.12±0.39
L7	I	23.66±2.64*	22.66±1.52	7.25±1.06	6.12±0.74
	II	7.66±1.05*	5.33±0.76	1.87±0.54	2.0±0.53

Results are expressed as the number of NADPH-diaphorase-reactive neurons per section in superficial laminae (I–II) in cervical (C3–C5) and lumbar (L5–L7) segments of the spinal cord. Asterisks indicate significant differences in the mean numbers±S.E.M. of labeled cells on the ipsilateral side in capsaicin-injected cats ($n=6$) vs. ipsilateral vehicle-injected animals ($n=3$), *$P<0.05$.

cord, thus being partly responsible for the "second wave" of *c-fos* expression (Harris, 1998). Most Fos-immunoreactive neurons are localized in the marginal zone (laminae I, IIo), neck (laminae V, VI) of the dorsal horn and intermediate zone (lamina VII) close to the area around the central canal (lamina X), that partially overlapped with the sites of termination of high-threshold muscle afferents in cat (Mense and Prabhakar, 1986; Williams et al., 2000). In this study, after intramuscular capsaicin injection, the majority of Fos-immunoreactive neurons were demonstrated ipsilaterally in lamina I of the C1–C5 segments, constituting ~40% of their total number (Fig. 3). The current data are consistent with the finding that significant projections of muscle nociceptive afferents terminate in lamina I (Mense and Craig, 1988), and terminals that express the vanilloid receptors TRPV1 are prominent in laminae I and II (Valtschanoff et al., 2001). A high density of Fos-immunoreactive neurons in laminae I and V was previously shown to occur in response to skeletal muscle fatigue, which was associated with the activation of high-threshold capsaicin-sensitive muscle afferents in rat (Pettorossi et al., 1999; Pilyavskii et al., 2001).

As described in Results, Fos-immunoreactive neurons in lamina I of the cervical and lumbar segments are presented as giant (>10 μm) or small (<10 μm) labeled nuclei. These giant neurons, so-called *Waldeyer cells*, are sources of the spino-parabrachial tracts, which transfer the nociceptive signals to the limbic structures involved in the affective, emotional or autonomic responses to noxious stimulation. One of the most unique features of the marginal giant cells

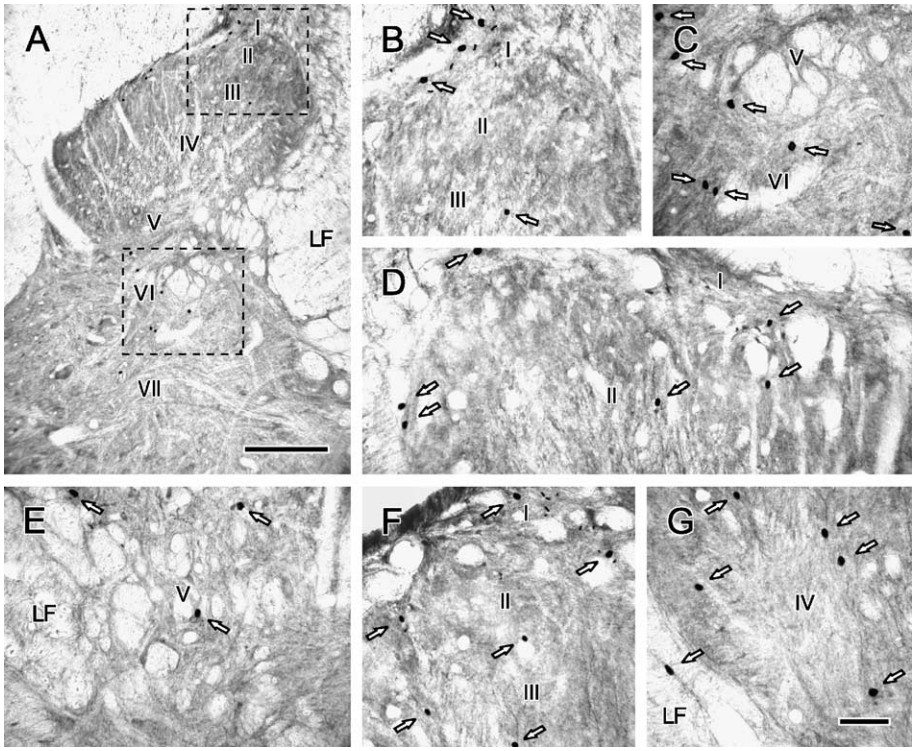

Fig. 4. Distribution of Fos-immunoreactive neurons in the ipsilateral (A–C) and contralateral (D–G) sides of the lumbar spinal cord after capsaicin injection into neck muscles. Note the bilateral distribution of labeled neurons (white arrows) and staining of glial-like cells (small reactive nuclei scattered close to the Fos-immunoreactive nuclei) in the dorsal horn (laminae I–VI) and intermediate zone (lamina VII) of the L3 (A–C), L5 (D–E), and L7 (F, G) segments. The dashed boxes in (A) delimitate the areas corresponding to the dorsolateral part (laminae I–III), and base (lamina VI) of the dorsal horn (B, C). LF, lateral funiculus. Scale bars: 200 and 50 μm for (A) and (B–G), respectively.

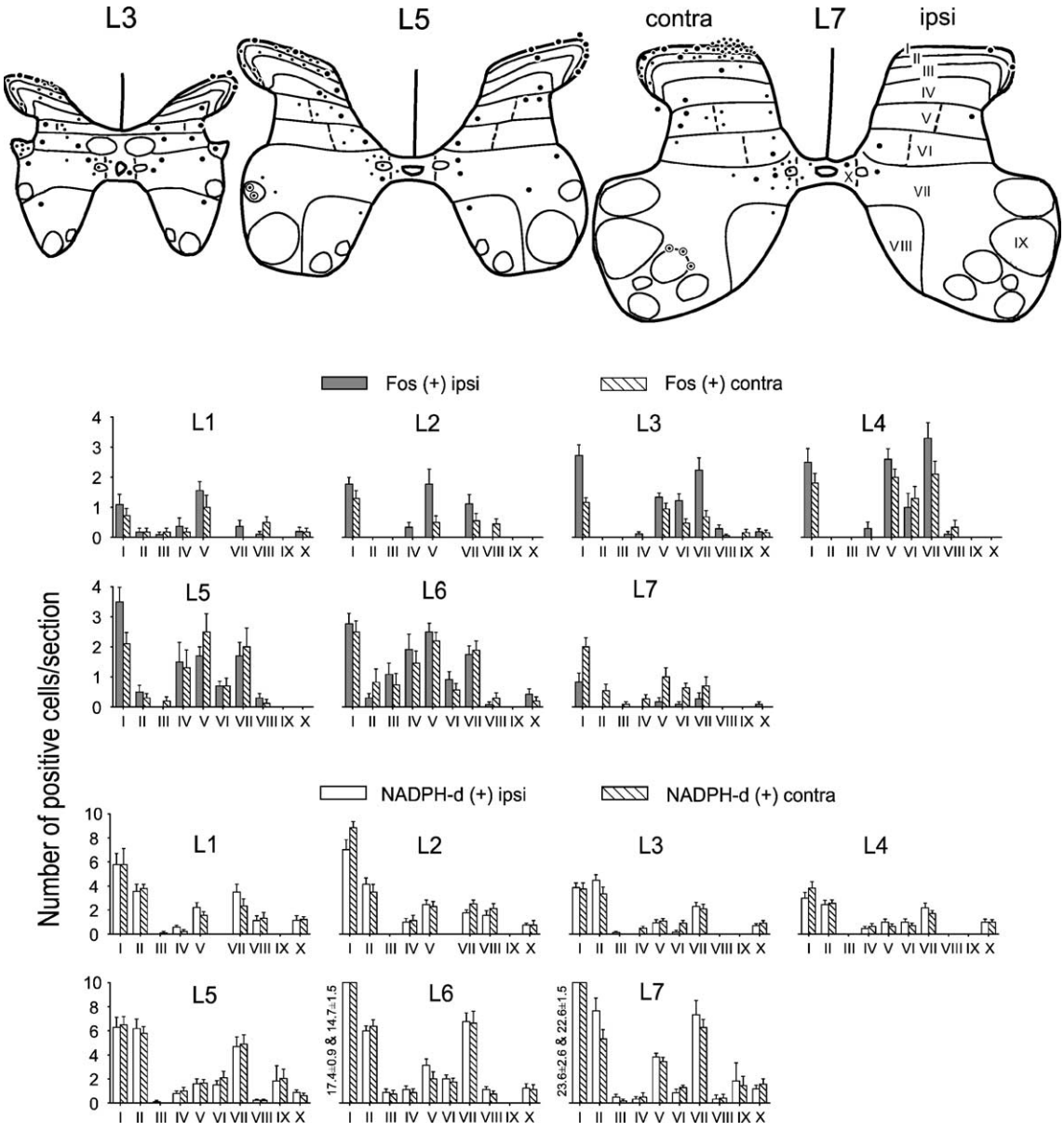

Fig. 5. Drawings (upper row) showing co-distribution of Fos-immunoreactive neurons and NADPH-diaphorase-reactive cells, and bar graphs (lower panel) of their mean numbers in the ipsi- and contralateral sides in the L1–L7 segments after capsaicin injection into neck muscles. Note NADPH-diaphorase-reactive neurons (circles) in motor nuclei. Mean numbers (±S.E.M.) of stained cells per section were defined in a group of six treated cats. Same designations as in Fig. 3.

is the very high density of GABAergic inhibitory input to their cell bodies and dendrites (Puskár et al., 2001). Moreover, it was shown earlier that, after noxious stimulation of hindlimb in the rat; a lot of Fos-immunoreactive neurons in the superficial laminae were identified as GABA or glycinergic inhibitory neurons (Todd et al., 1994). Recently, strong evidence for the important role of presynaptic inhibition in the modulation of motor output during muscle fatigue has been demonstrated (Pettorossi et al., 1999; Kalezic et al., 2004a; Kostyukov et al., 2005). The registration of Fos-immunoreactive neurons in the intermediate zone and the ventral horn of the lumbar cord after capsaicin injection supports the notion that premotor interneurons located there could be strongly activated during tonic pain of the dorsal neck muscles. In the study, *c-fos* expression was found in a small number of motoneurons in lamina IX at the C2–C7 level (Fig. 3). However, in non-anaesthetized rats, prolonged walking (1 h) can entail considerable labeling of motoneurons (Jasmin et al., 1994). The revealed capsaicin-induced effects on *c-fos* expression in the lumbar spinal cord are in compliance with the modulation of neuronal activity in the lumbar spinal cord from cervical spinal cord (Jones, 1998), thus displaying the plasticity of nociceptive processing along the cervico-lumbar extent.

Recently, it has been shown that neuronal NOS are upregulated shortly after intradermal capsaicin injection (Wu et al., 2001). The injection of capsaicin into joint or

muscle produces a deep tissue pain that typically referred to superficial structures, whereas cutaneous pain is not usually referred. Moreover, capsaicin injected into muscle resulted in a long-lasting mechanical allodynia compared with injection of capsaicin into skin (Gronroos and Pertovaara, 1993; Witting et al., 2000; Sluka, 2002). Our finding of bilateral increase in the numbers of NO-generating neurons within the lumbar spinal cord is the first demonstration that NO may be involved in the initiation of secondary pain-related events in distant muscle groups (referred pain). The short delay in up-regulation of NOS expression in lumbar segments may be related to the initial persistent expression of transcription factors linked to the inducible form of NOS induction (Herdegen and Leah, 1998). In the present model of muscle pain NOS-containing neurons did not reveal Fos-immunoreactivity. The double labeling was not found in the spinal neurons (Pilyavskii et al., 2001), however, it demonstrated well in medullar neurons after skeletal muscle fatigue (Maisky et al., 2002).

Glial cells are now known to reciprocally communicate with neurons, and they may play a more direct role in modulating neuronal transmission than is imagined, and they are sources of pronociceptive mediators (Watkins et al., 2001). During spinal nociceptive processing, glial cells release a variety of neuroactive substances, including NO. Activation of glial cells enhances the release of substance P and excitatory amino acids from high-threshold primary muscle afferents in the dorsal horn (Oka and Hori, 1999). Findings of Fos-immunoreactive glial nuclei, especially within the superficial dorsal horn (laminae I and IIo) of the cervical and lumbar segments (Figs. 1B, E, H and 4B, D, F), reflect capsaicin-related inflammatory processes in the periphery and the spinal cord. It is possible that activation of glial cells is one of the mechanisms involved in the creation of extra-territorial pain and/or mirror-image pain.

The spread of capsaicin-induced pain sensations to other regions might in part be explained by inter-segmental reflexes. It was shown earlier that pain signals from chemosensitive afferents of masticatory muscles could activate the fusimotor neurons supplying the neck muscles in humans (Arima et al., 2000), and that signals from high-threshold neck muscle afferents induced substantial changes in the responses of lumbar γ-motoneurons in cat (Ellaway and Murthy, 1984). Neck muscle fatigue and pain, which most probably activate small-diameter afferents of group III and IV (Darques and Jammes, 1997), also affect upright posture (Michaelson et al., 2003; Schieppati et al., 2003; Gosselin et al., 2004). As compared to normal subjects, patients suffering from chronic work-related neck pain or whiplash disorders show larger sway areas during quiet stance, and reduced stability during more challenging postural tasks, such as standing on one foot or on feet in tandem arrangement, as well as reduced stability vs. postural perturbations (Michaelson et al., 2003). The various sensory systems may interact in a complicated way, involving interwoven feedback loops. For example, nociceptive afferents of group III and IV affect fusimotor neurons, which in turn change muscle spindle afferent discharge, that could lead to alterations in proprioception and motor control and, ultimately, spreading of pain (Windhorst, 2003). The spino-cerebro-spinal loops were shown to involve in the transmission of influences from neck nociceptors to the lumbar spinal cord (Urban and Gebhart, 1999; Millan, 2002). At the same time, propriospinal descending routes (Menétrey et al., 1985) would provide a more direct neuroanatomical basis for the irradiation of cervical pain toward the lumbar spinal segments.

In conclusion, received data show that activation of vanilloid receptors in the dorsal neck muscles by capsaicin leads to development of the distinctive patterns of *c-fos* expression and NADPH-diaphorase reactivity in the cervical and lumbar spinal cord. The revealed changes in neuronal and glial activation are suggestive of plasticity in segmental circuitries in response to nociceptive input from the dorsal neck muscles that could be an important mechanism in initiating the adaptive inter-segmental reflexes in the spinal cord.

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